IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
(Attorney Docket No. AM101333)

In re Patent Application of:	)	Appln. No.: 10/796,925
	)	Confirmation No.: 3270
WUMIN LI *et al.*	)	Customer No.: 25291
	)	Group Art Unit: 1645
Filed: 03/10/2004	)	Examiner: Lakia J. Tongue
	)	
For: ADJUVANTED BOVINE VACCINES	)	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

Dear Sir:

I, WUMIN LI, declare and say as follows:

THAT I am the same WUMIN LI who is named as co-inventor with HSIEN-JUE (STEVE) CHU in the above-referenced application for United States Letters Patent;

THAT I currently hold the position of Senior Manager, Biological Research and Development at Fort Dodge Animal Health in Fort Dodge, IA since 2003 to present date;

THAT I previously held the position of Manager, Biological Research and Development at Fort Dodge Animal Health from 1999 to 2003 and the position of Project Leader as a research scientist at Fort Dodge Animal Health from 1995 to 1999;

THAT I had been employed as a research scientist at Pfizer Animal Health, Groton, Connecticut from 1993 to 1995;

THAT I received the degrees of Masters of Science in Veterinary Science in 1989 and Doctor of Philosophy in Veterinary Science in 1993 from the University of Wisconsin-Madison, Department of Animal Health and Biomedical Sciences, Madison, WI;

THAT I am familiar with the above-said application and do understand the Official action of March 8, 2007 pertaining thereto;

THAT the following experiment was conducted at my behest and on my behalf to demonstrate that the *E. coli* O157:H7 vaccine formulation of the present invention provides an unexpectedly safe injectable formulation that elicits a markedly effective immune response.

Experiment

Evaluation of Serological Response and Safety of *E. Coli* O157:H7 Vaccine in Cattle

A side-by-side comparison study was performed to evaluate the serological response and safety of test *E. coli* O157:H7 vaccines in cattle following vaccination. Control Group 1 remained unvaccinated during the experiment. Only the adjuvant varied between the two test vaccine groups. Group 2 was vaccinated with an *E. coli* O157:H7 vaccine containing a standard adjuvant that consisted of aluminum hydroxide (art-recognized as a conventional vaccine adjuvant). Group 3 was vaccinated with an *E. coli* O157:H7 vaccine containing the novel metabolizable oil adjuvant of the present invention that comprised aluminum hydroxide and SP Oil. As used in the experiment and the application, the term "SP Oil" designates an oil emulsion comprising a polyoxyethylene-polyoxypropylene block copolymer, squalane, polyoxyethylene sorbitan monooleate (Tween 80, an emulsifier) and a buffered salt solution.

Twenty-four healthy mixed breed cattle obtained from commercial sources were used in the study. Their age range was 6 – 12 months at first vaccination, and both male and female animals were used. The cattle were group housed in housing meeting applicable animal welfare regulations. Water and food were available *ad lib.* All animals were treated as deemed necessary by the plant veterinarian after consultation with the study director. Treatments before and during the study were documented. Animals requiring antibiotics or potentially immunosuppressive drugs were removed from the study.

Vaccine compositions were formulated and tested for sterility and laboratory animal safety as specified in 9 C.F.R. §§ 113.26 and 113.33. Vaccines were stored at 2-7°C. Calves were randomly divided into groups of six animals each. Group 2 was vaccinated with the test vaccine containing the conventional adjuvant. Group 3 was vaccinated with the test vaccine containing the unique metabolizable oil adjuvant in accordance with the present invention. Group 1 was held as unvaccinated controls. Calves were vaccinated with a 2 mL dose of the appropriate vaccine by the subcutaneous route. A second dose was administered in 3-4 weeks, and a third dose was administered after a further 3-4 weeks. Calves were bled at the time of the first and second dose, and then weekly thereafter until four weeks post third vaccination. Each serum sample was evaluated for antibody response.

Serum analysis was done by statistical methods to determine differences in antibody response. ELISA titers were determined to assess vaccine response, and results were averaged. Injection sites were observed for three days following each vaccination. If any injection site reactions were seen, the cattle were then observed up to 14 days post vaccination or until the reaction had dissipated. Injection site reactions were measured in three dimensions (length, width and height). A daily reaction score was calculated by L x W x H. Total reaction scores were analyzed by Mann Whitney Rank Sum. The level of significance was set at $p<0.05$.

ELISA titer results are shown in the following Table 1.

Table 1: ELISA Titer Results of Serology Testing

Vaccine Group*	**Calf Number**	**0 Days Post First Vaccination**	**14 Days Post Third Vaccination**
1	283	640	1280
1	291	640	640
1	367	640	640
1	368	640	640
1	369	640	640
Average (1)		640	735
2	389	640	640
2	277	640	640
2	292	2560	2560
2	379	320	640
Average (2)		735	868
3	390	640	1280
3	384	1280	2560
3	294	320	1280
Average (3)		573	1184

* Vaccine Groups: Group 1 = control; Group 2 = standard adjuvant; and Group 3 = SP Oil/AlOH adjuvant of present invention.

Injection site reactions are shown in the following Table 2.

Table 2: Reaction Scores Assessing Injection Site Reactions

Vaccine Group*	-1dpv2**	0dpv2	1dpv2	2dpv2	3dpv2	4dpv2	5dpv2
1	0	0	0.0	0.0	0.0	0.0	0.0
2	0	0	68.4	58.0	31.9	30.8	19.0
3	0	0	25.4	61.5	43.3	52.1	61.3

Vaccine Group*	6dpv2	7dpv2	10dpv2	11dpv2
1	0.0	0.0	0.0	0.0
2	9.8	10.1	6.6	1.5
3	24.9	15.3	1.2	2.8

* Vaccine Groups:
Group 1 = control;
Group 2 = standard adjuvant; and
Group 3 = SP Oil/AlOH adjuvant of present invention.

** Heading definitions:
-1dpv2 = assessment of injection site on day before second vaccination
0dpv2 = assessment of injection site on day of second vaccination
1dpv2 = assessment of injection site one day post second vaccination
2dpv2 = assessment of injection site two days post second vaccination
3dpv2 = assessment of injection site three days post second vaccination
4dpv2 = assessment of injection site four days post second vaccination
5dpv2 = assessment of injection site five days post second vaccination
6dpv2 = assessment of injection site six days post second vaccination
7dpv2 = assessment of injection site seven days post second vaccination
10dpv2 = assessment of injection site ten days post second vaccination
11dpv2 = assessment of injection site eleven days post second vaccination

The above ELISA titer results in Table 1 demonstrate that the animals of Group 3 surprisingly showed significantly enhanced immunogenic responses over those of Group 2 and the control group based on the levels of the ELISA titers fourteen days post third vaccination.

Insofar as the variation in a few titers is concerned, it is explained that some abnormalities are to be expected under the circumstances of working with live animals. Applicants utilized a good control facility and exercised care during the experiment. However,

E. coli is ubiquitously found. The calves that had a high titer before injection (calf # 292) or had an increased titer without treatment (calf # 283) might have been infected with another *E. coli* strain and peaked as a cross-reaction despite the attempt to keep the controls clean of infection; or perhaps one of the calves had been exposed accidentally to *E. coli* O157:H7. Any practitioner who works in the veterinary field with cattle would appreciate these and other possible reasons for certain titers to be anomalous.

The more important value of the titers in Table 1 is seen in the overall average of each group of animals involved in the study, namely, a significantly improved titer of 1184 for those treated by the vaccine formulation of the invention (Group 3) as compared to 868 for those animals treated by the vaccine containing the conventional adjuvant (Group 2) and further compared to 735 for the controls (Group 1). By and large, the controls stayed at the baseline titer of 640 (a normal range) and the titers of the conventionally adjuvanted vaccine remained the same over time. Yet, unexpectedly, it was observed that the vaccine composition of the invention (metabolizable oil plus aluminum hydroxide) demonstrated a significant improvement in titers over the standard adjuvant (aluminum hydroxide) used in the comparative vaccine.

Considering the significantly higher immunogenic responses in Group 3 of the present invention, the above reaction scores in Table 2 revealing similar rates of injection site reactions to the two test vaccines on comparison and the noteworthy observation of no major reaction after inoculation with the vaccine of the invention were not anticipated. With all vaccinations, a little lump is to be expected when the active ingredient is released slowly from the site of depot administration but vaccines that give a significantly higher immune response typically cause a much greater site reaction that is deleterious to meat quality. Because severe lumps usually form from potent vaccines, it was presumed that the vaccine composition having the higher host immune response would cause a greater adverse reaction, which would adversely impact the meat quality of the animals sold for food consumption. It was unexpected, therefore, to observe that the size of the reaction lump of the vaccine of the invention was the same as a traditional vaccine formulation and the animals of both test groups displayed minimal, normal injection reactions at the vaccine administration sites.

As can be seen by the above experiment, the results establish that the vaccine of the present invention is able to stimulate the host immune system and elicit a potent immune response against *E. coli* O157:H7. The data showed that the vaccine formulation of the present invention provided the greatest overall serological titers to *E. coli* O157:H7 as compared to a standard vaccine formulation of the art and the placebo group (the control). Since there is a direct correlation between antibody titer and vaccine efficacy, the superior host serological response clearly establishes that the vaccine containing the metabolizable oil adjuvant of the invention would beneficially induce active immunity against *E. coli* O157:H7, prevent colonization of *E. coli* O157:H7, provide bactericidal effect and, consequently, reduce shedding in cattle.

The results also substantiate that the *E. coli* O157:H7 vaccine composition containing the metabolizable oil adjuvant is safe for immunizing food animals. The unpredictable reaction scores in light of the serological testing prove that the vaccine of the invention provides beneficial biological activity and a practical advantage over the traditional vaccine formulation in being highly effective yet safe on administration to cattle.

I, the undersigned, declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with knowledge that willful, false statements, and the like so made, are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 06 July 07 By: Wumin Li

WUMIN LI, Ph.D.